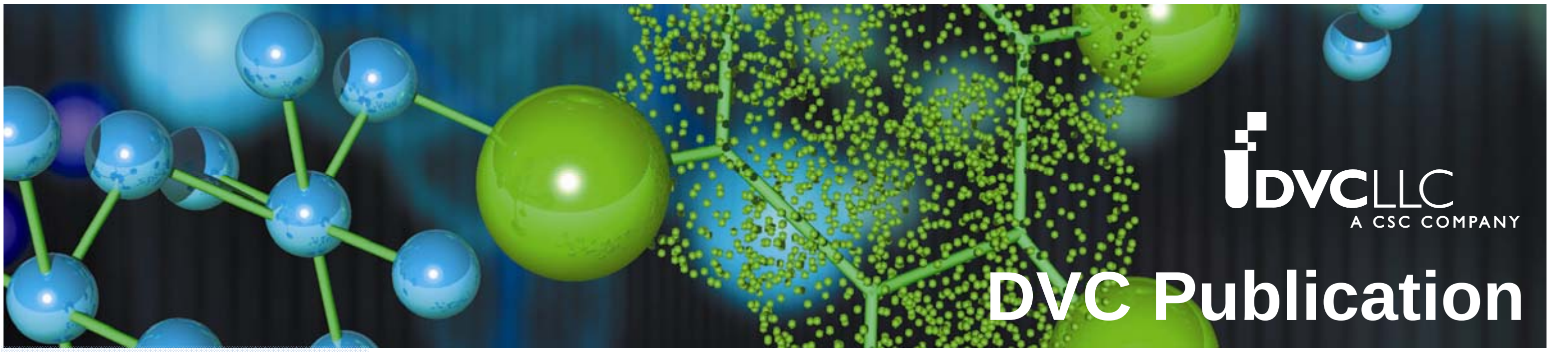

Novel Vaccine Delivery – Edible Vaccines (November 2005)

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TechWatch Report

Edible vaccines provide an example of a novel strategy that has the potential to combine the innovations in medical science and plant biology to create affordable pharmaceutical products. There are several advantages to the use of plant-made vaccines (Walmsley et. al., 2000). Currently, most of the available vaccines are inactivated or attenuated strains of viruses or bacteria that are delivered by injection. These low-virulence pathogens can cause severe disease in immunocompromised individuals. The use of subunit vaccines, proteins of the pathogen that can stimulate the immune response directed at the intact virus or bacteria have become increasingly popular to circumvent this problem. Cloned antigen genes can be transferred to bacteria or yeast where they are expressed and purified for injection. However, it is now theoretically possible to use transgenic plants as a viable alternative to the more expensive cell culture systems for the production of recombinant subunit vaccines.

In theory, the benefits of such a system are enormous. Increasing the scale of production would simply be a matter of increasing the acreage of the stable transgenic line, and would not require large investments in hardware or culture media. Edible vaccines would also offer an oral delivery system that would preclude the costly purification process required for injectable vaccines (although it would be feasible to purify pharmaceutical products from plant material). Furthermore, oral delivery is more likely to generate a mucosal immune response than injectable vaccines. The mucosal immune response is characterized by the production of secretory IgA (sIgA) and its specific transport across the epithelium to the mucosal surface. sIgA acts as a first line of defense against pathogen colonization and infection by binding bacterial cells and virus particles and thus preventing their specific interactions with receptors on the mucosal cell surface. Parenteral (injectable) delivery only rarely results in strong sIgA responses. Ingestion of plants expressing appropriate vaccine antigens could result in specific protective

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mucosal sIgA and serum IgG responses against microbes that display these antigens on their surfaces.

Edible vaccines have additional advantages. Plant recombinant expression systems are much less likely to harbor pathogenic microbes (adventitious agents) or prions than are mammalian cell systems. Plant cells with rigid walls provide protection for orally administered vaccines from the acidic environment of the stomach. Plant-made vaccines also can be delivered to the patient without the need for syringes which might become contaminated with multiple use, and can be administered without the need for clinical oversight that would be necessary for injectables, a benefit in underdeveloped countries. Finally, expression of vaccines in plant tissue, such as seeds or grain, provides a heat stable environment, disposing of the need for cold chain between manufacturer and patient.

Vaccine antigens can be produced by either stable genetic transformation, achieved with the transforming pathogen *Agrobacterium tumefaciens*, or transient expression. Transient expression uses a recombinant plant virus that carries the vaccine gene and, by systemic infection, causes the plant to express the antigen.
Because edible vaccines target the mucosal immune response, the first vaccines under development were those that inhibit infections of the gastrointestinal tract (Tacket, 2005). These would include viruses of the Norovirus family (i.e., Norwalk virus in humans), enterotoxic *E. coli*, and cholera. The first plant vaccines were developed in tobacco since this species is easy to transform and regenerate. However, other more suitable plants have replaced tobacco, such as potato, tomato, lettuce, spinach, carrot, and corn (Tacket, 2005). The first plant vaccine against enterotoxic *E. coli* was based on the highly immunogenic heat-labile enterotoxin (LT) of *E. coli*. LT is composed of two subunits, A, the ADP ribosyl transferase activity, and B, five immunogenic binding subunits (LT-B) that bind to the GM1 ganglioside present on epithelial cell surfaces. Immune responses to LT-B can provide short term protection to infection from LT-producing *E. coli*.

The cloning and expression of LT-B in plants has been described (Haq et. al., 1995). Two expression vectors were constructed. The first was comprised of an expression cassette for kanamycin resistance for selection and an LT-B expression cassette with a eukaryotic promoter. The second also included DNA encoding the polypeptide SEKDEL for endoplasmic reticulum retention of the protein. Individual transformants of tobacco and potato plants showed varying amounts of gene expression, resulting from random nuclear insertion of the transferred DNA. The first construct showed maximum amounts of protein accumulation of up to 5 µg per gram of total soluble tobacco leaf protein and up to 30 µg per gram of soluble protein in potato microtubers. The second construct showed increased expression, with up to 14 µg per gram of tobacco leaf protein and 110 µg per gram of potato microtuber protein, representing a high of 0.01% of total protein. The microsomal retention signal, SEKDEL, caused cellular compartmentation of the fusion protein, thus facilitating oligomerization. A protein band from extracts of the first construct was observed on SDS-PAGE gels that comigrated with bacterial derived monomeric LT-B. A slightly larger protein band was seen from extracts of the second construct. The second construct was used in immunogenicity experiments with mice. BALB/c mice were given a crude extract from tobacco leaves by gavage. A second group received bacterial expressed LT-B. Serum and mucosal samples were collected and antibody titers determined by ELISA for rLT-B specific antibodies. Amounts of serum antibodies were similar in the two groups. Mucosal samples also showed antibodies to LT-B. The protective nature of these antibodies was shown in toxin neutralization assays. When mice were fed with potato tubers from transgenically expressing plants, serum and mucosal samples were positive for LT-B antibodies.

The low level expression of LT-B in tobacco and potato plants is problematic. Therefore, efforts were made to optimize the expression in plants (Mason et. al., 1998). A synthetic LT-B coding sequence modified to contain plant-preferred codons and to eliminate spurious mRNA processing signals was constructed. The synthetic LT-B gene maintained the amino acid sequence of the native gene. The highest accumulation of LT-B was shown to be 1.9% of total soluble leaf protein. This increase in expression was due in part to increased mRNA levels as compared to the native gene. Also it is likely that synthetic LT-B mRNA is translated more efficiently due to the use of plant codons. However it was shown that high level expression of LT-B had a growth-inhibiting effect on plant cells, resulting in poor tuber development. The potato tubers were also shown to be immunogenic in mice. The mice immunized with synthetic LT-B transgenic potato tubers were protected against challenge with toxin.

Still, the level of expression of the antigen is low for eliciting a strong immune response. Transgene expression could be increased if the antigen were expressed from the chloroplast genome (Maliga, 2002). Up to 45% of total soluble protein could be expressed when the protein was transferred to the chloroplast. Other advantages to chloroplast transformation include maternal inheritance (which would eliminate the problem of contaminating wild-type species through pollen), specific gene targeting, high copy number, removal of selectable markers (which could make up 10% of the total soluble protein), the potential use of operons and retention of the gene product in the plastid. A strong disadvantage is that proteins would not be glycosylated.

The success of LT-B expression in potato tubers and immunization in mice led to the first clinical trial in 1997 (Tacket et. al., 1998). In the double-blind study, the volunteers ate either 50 or 100 grams of raw LT-B potatoes containing 375 or 750 µg LT-B or non-transgenic placebo potatoes, administered in three weekly doses. Ten out of 11 subjects in the LT-B group developed at least four-fold increases in levels of serum LT-neutralizing anti-LT-B IgG, which persisted in sera up to 38 days after the final dose. Five of 10 volunteers showed increases in anti-LT-B IgA in stool samples. There was no correlation between responders and dosage level, which could reflect variations in antigen content in the potatoes. This study proved that ingestion of transgenic plant material could be used effectively to stimulate potentially protective antibody responses in humans.

<u>The use of plant vaccines against biological warfare agents.</u>

Advances have been made on the selection of biological warfare agents in transgenic plants. Aziz et. al. (2002) have cloned the gene for Protective Antigen (PA) of *Bacillus anthracis* under the control of a eukaryotic promoter. The resulting construct was mobilized into *Agrobacterium tumifaciens* which was used to transform tobacco plants. The PA gene was shown to be integrated into genomic DNA by PCR analysis. Total soluble protein was extracted from tobacco leaves was subjected to immunoblot analysis and a band of 83 kDa, the expected weight of PA, was observed. Cytotoxicity experiments were carried out on total soluble protein obtained from transgenic tobacco leaves. It was shown that plant expressed PA, along with lethal factor, was able to lyse indicator cells and showed biological activity comparable to that of native

PA. This is the first step in making an edible plant vaccine against anthrax. Similar work could be performed with subunit vaccines for other biowarfare agents.

Although there has been success in the development of edible vaccines, some problems still exist (Vermij, 2004). Regulatory agencies will not approve vaccines with variable dosing. Also there is the possibility that edible vaccines might induce oral tolerance instead of eliciting an immune response. This issue must be studied for each case individually because protein characteristics vary greatly. Another issue is the question of humoral versus cell-mediated immunity. There has been little study of cytotoxic T-cell responses, which are important for protection against many intracellular pathogens.

Plant-derived vaccines show great promise for increasing the ease and decrease the cost of vaccine delivery to humans and animals. However there is a concern in the general public about genetically modified crops contaminating the food supply, and consumer groups have called for a ban on using food crops to produce pharmaceuticals. Producing veterinary vaccines first may overcome some of these fears. If the argument can be made that edible vaccines may save the lives of millions in developing countries, the public should be more receptive to this exciting new field.

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